Effect of life style intervention on markers of oxidative stress, inflammation and cellular aging

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Abstract— *There are 3 markers of cellular aging – oxidative stress, DNA damage and decline in telomerase activity. A hectic life style, psychological stress, increased smoking, drinking and other such habits leads to supra physiological free radical levels. Hence this study was planned to evaluate role of life style intervention (Yoga and Meditation) by investigating various oxidative stress markers such as 8-hydroxy-2'-deoxyguanosine (8-OHdG)* and *Reactive Oxygen Species (ROS), psychological markers of stress cortisol, β- endorphins, inflammatory markers like IL-6 and markers of cellular aging as Telomere length and Telomerase activity. For this study 75 healthy volunteers enrolled in Integral Health Clinic (IHC) of All India Institute of Medical Sciences, New Delhi. Venous blood samples were collected at after each intervention (Day 0, day 10 and day 90). Various biomarkers such as plasma cortisol, β-endorphins, IL-6, ROS and 8-OHdG levels were measured. Telomerase activity and telomere length were also assessed. It was that there was significant reduction in level of oxidative stress markers (ROS and 8-OHdG), psychological stress marker (cortisol) and inflammatory marker (IL-6). Anti-aging marker (telomerase) and β-endorphins level was also observed significantly increased in these subjects after10 days and 90 days of yoga/meditation practice. There was also increase in telomere length after 90 days of yoga/meditation practice.So that it can be concluded that inclusion of yoga/meditation in daily life if very beneficial, so it should be adopted as healthy lifestyle.*

Key words**: **Aging, Telomerase, Telomere, Psychological stress, Oxidative stress

1. Introduction

Oxidative stress is a distress that generates a sea of chemical and hormonal reactions in the body by free radicals and it damages both, mitochondrial and nuclear DNA.[1] The result of such a response to stress is physiological support for adaptive behaviors such as "fight or flight".[2] As a part of the adaptive response to this oxidative stress, various body systems such as the immune system and reproductive system may be affected. The modern lifestyle is known to lead to psychological stress which results in increased production of inflammatory and oxidative stress markers which form the basis of several life style related diseases like cancers, infertility, hypertension and autoimmune diseases. [5,1]

Oxidative damage preferentially damages telomeric DNA and accelerates telomere loss, whereas antioxidants decelerate it. [3] Oxidative stress is an important modulator of telomere loss and that telomere-driven replicative senescence is primarily a stress response. Telomere shortening is counteracted by the cellular enzyme telomerase. [4] Telomerase adds telomeric repeat sequences to the chromosomal DNA ends, preserving not only telomere length, but also healthy cell function and long-term immune function. [5] As telomeres shorten after each cell division, therefore, rate of shortening and the telomere length are index of mitotic-cell age. [6] In human, reduction in telomere length is an ideal prognostic marker for disease risk and progression and for premature death.[7]

However, complementary and alternative medical therapies such as yoga and meditation are being increasingly used as adjuncts to modern medicine. Yoga's eminence for stress reduction has

fortified its popularity in recent years, and data from various randomized trials suggest that use of yoga and meditation reduces symptoms of anxiety and stress. [8,9] There are various types of yoga posture, one of which is *hatha* yoga, the most commonly used in western part of the world, combines breath control and meditation[10] A recent report consist of heart failure patients trained for two-months hatha yoga, showed 22% reduction in levels of IL-6.[11] Furthermore, various lifestyle habits may significantly impact on inflammatory status of body. Such as, in obese people, due to increased level of IL-6 and TNF-α obesity is considered as a state of chronic inflammatory disease.[12] Therefore, one evident mechanism is provided by the fact that adipocytes are very much capable of producing and secreting IL-6 and TNF-α; in fact, up to 30% of IL-6 may be derived from adipose tissue.[13] Some other studies also suggest that by yoga and meditation the cortisol level may also reduce along with pro inflammatory cytokines.[14] Keeping above facts in mind, it was felt that there was an urgent need for a study which consist of fair number of subjects and can analyze all sets of biomarkers mentioned above including biomarkers for aging following interventions.

Although there have been a few studies to demonstrate the effect of yoga on oxidative stress[5], to the best of our knowledge, none has simultaneously measured the objective parameters of oxidative stress (ROS), 8-OHdG, Cortisol, IL-6, β-endorphin, telomere length and telomerase activity and it was done at baseline (day 0), at the end of interventions (day 10) and follow up (90 days). Therefore, this study was planned with the aim to investigate the effect of yoga/meditation on oxidative stress parameters, plasma levels of β-endorphin, IL-6, cortisol, telomere length and telomerase activity.

2. Methods

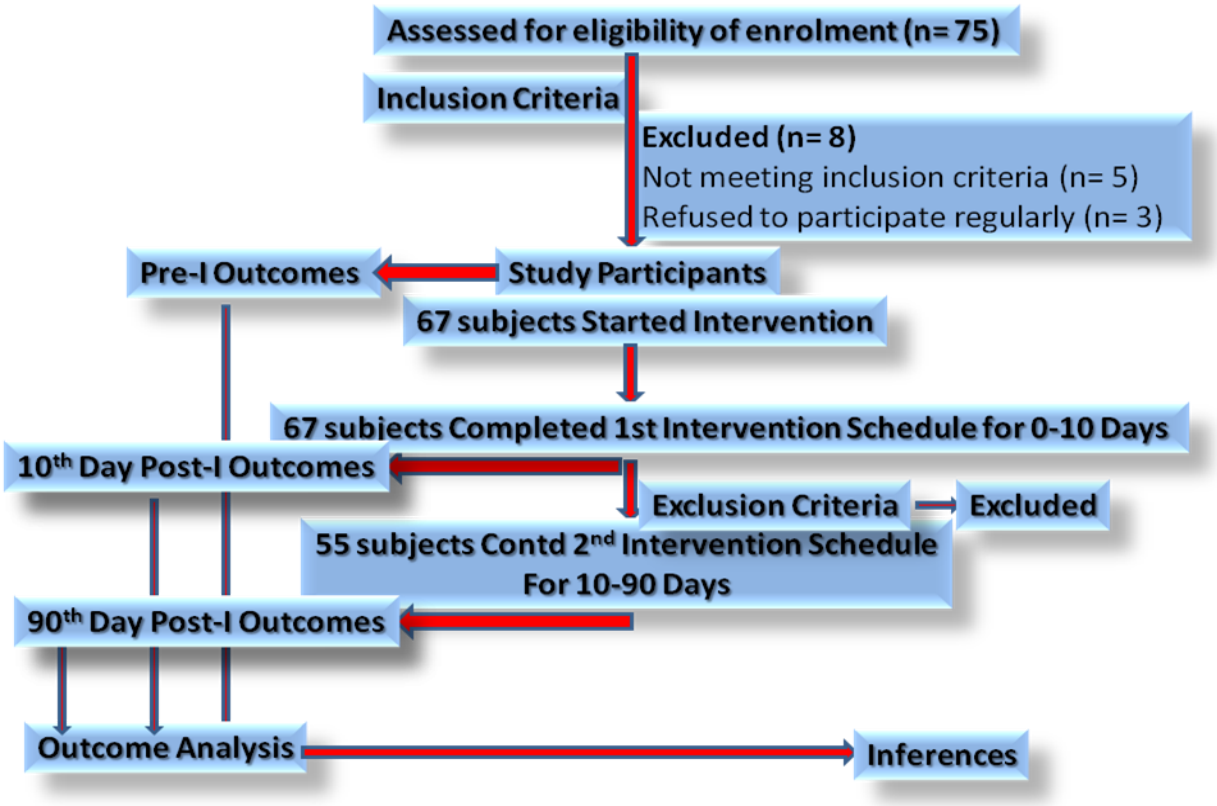
Study Participants: Seventy five healthy subjects of aged 30-60 years (38.38±16.19 years) volunteered were enrolled to join the program for fitness in November 2013. Of these, the subjects with any chronic diseases and mental stress who had a diagnosis made at different respective clinics were excluded from study. Out of these 75 subjects, ultimately only 55 subjects remain in the study who had completed 90 days fitness programme. (Fig.1). Key inclusion criterions were agreement of the subject to attend regular intervention at our Integral Health Clinic and exclusion criterions were physically challenged subjects and those who were on any type of medicines/antioxidants.

Lifestyle intervention: The informed consent was obtained by each participant the study was approved by the Institutional ethical board. The design of interventions were published previously in detail by our group.[5,6] But in short, the intervention program lasted for 2 hours each day and that was for 90 days, comprising theory and practice sessions. Generally, the program starts with an array of asanas consists of various postures and pranayama which was a typical breathing exercise.

Estimation of all programmed biochemical markers were done in fasting venous blood samples. Blood samples (5mL) were collected in heparin coated vials and centrifuged at 2000g for 15 minutes and plasma was preserved at -80ºC. The biochemical markers were assayed by commercial ELISA kits. Pre tasted the quality-control was validated for the biochemical markers and the methods used.

This fasting venous blood samples were taken on the day i.e. '0' day of starting intervention then on 10^{th} day on completion of 1^{st} schedule of intervention. After that who were eligible and want to participate in next schedule of intervention i.e. from 10^{th} day to 90^{th} day, were continued in study. Fasting venous blood samples was taken again on the 90^{th} day i.e. on completion of 2^{nd} schedule of intervention.

Flow chart of methodology



For outcome venous blood sample was taken on '0' day, 10th day and 90th day of intervention. Following levels were assessed from taken fasting venous blood sample on '0' day, 10th day and 90th day of intervention and was compared.

Measurement of telomerase activity: Telomerase level was determined using telomerase assay kit (Roche, Switzerland) as per manufacturer's protocol. In brief, Peripheral Blood Mononuclear Cells (PBMCs) were obtained by gradient density centrifugation by use of Ficoll-Paque (GE Healthcare, Piscataway, NJ, USA). PBMCs were stored at –80°C until used for assay. The levels of telomerase were measured at each intervention (baseline, 10 days and at 3 months).[7]

Telomere length (T/S Ratio): Blood DNA was extracted using a QIAamp DNA mini kit (Qiagen Ltd.,Toronto, ON, Canada) as instructed by manufacturer's. Telomere length was determined by real-time polymerase chain reaction (qPCR), as previously described.[8] Briefly, two PCRs were done for each sample, one to determine the cycle threshold (Ct) value for telomere (T) amplification with the telomeric primers (Forward 5'-CGG TTT GTT TGG GTTTGG GTT TGG GTT TGG GTT TGG GTT-3' and reverse 5'- GGC TTG CCT TAC CCT TACCCT TAC CCT TAC CCT TAC CCT-3') and the other to determine the Ct value for the amplification of a single-copy (S) control gene 36B4 (Forward 5'- ACT GGT CTA GGA CCCGAG AAG - 3' and reverse 5'- TCA ATG GTG CCT CTG GAG ATT - 3'). Each sample was run in triplicate, and each qPCR was done using 20 ng of DNA sample in 25µl final reaction volume. Mean Ct values were used to calculate the relative telomere length using the telomere/single copy gene ratio (T/S).[8] All PCRs were performed on the CFX96 (Bio- Rad Thermal Cycler, USA).

8-Hydroxy-2'-deoxyguanosine Assay: Plasma 8-OHdG was measured using ELISA Kit (Cayman Chemical, USA) as per manufacturer's protocol and also previously described.[15,16] In brief, the test utilizes an anti-mouse IgG-coated plate and a tracer consisting of an 8-OHdG conjugate. Absorbance

was measured at 450 nm using a micro plate Reader (Biotehnique, USA). The results were calculated with the Cayman data analysis system.

Estimation of Reactive Oxygen Species: ROS level estimation in whole blood done by estimating the luminol-dependent chemiluminescence with luminometer (Sirius, Berthold Detection Systems GmbH, Germany) in the integrated mode for 10 minutes. In four hundred micro-litters of blood, ten micro litters of luminol (5-amino-2, 3,-dihydro-1,4-phthalazinedione; Sigma, USA),(5 *mM)* in dimethyl sulfoxide (DMSO), was added as a probe. A negative control was prepared by adding 10 μL of 5 *mM* luminol to 400 μL of PBS. The results were expressed as RLU/min/10^4 Neutrophils.[16]

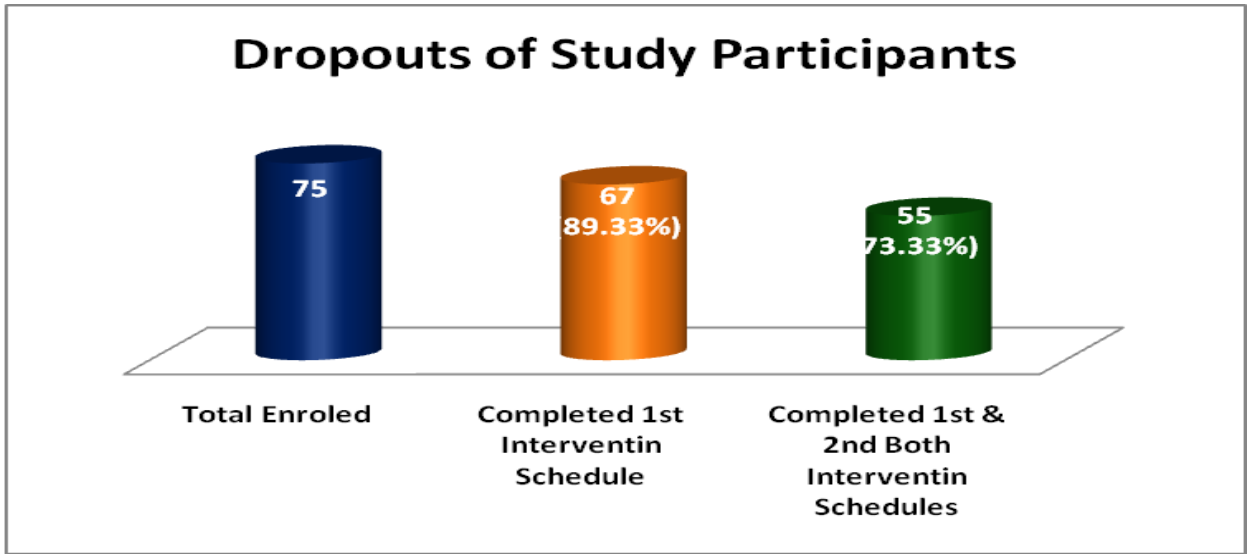
Estimation of biochemical markers: Fasting venous blood samples (5 mL) were collected in heparinized vials after each intervention. Samples were centrifuged (3000g, 10 minutes, 4ºC) and plasma was stored at - 80ºC until analyzed. The biochemical markers level were assayed using commercially available ELISA kits for plasma levels of β-endorphin (Phoenix Pharmaceuticals, Inc.), cortisol (DRG Diagnostic, Germany),IL-6 (Gen-Probe, Diaclone Diagnostic, France). Quality-control for each biomarkers was taken and methods were validated.[16]

Statistical analysis: Statistical analysis was performed using the SPSS 15.0 software trial version (SPSS Inc., Chicago, IL, USA). Paired and unpaired corresponding differences in various experimental parameters were examined using one-way repeated-measures analysis of variance (ANOVA), $p<0{\cdot}05$ was used as the level of significance.

3. Results

Initially 75 apparently healthy subjects of aged 18-60 years were enrolled for the study but out of these 8 subjects were excluded from the study as 5 subjects were found not fit for intervention and 3 subjects had refuse to take part in intervention regularly. So, to start with 67 subjects were entered in 1st schedule of intervention. But after completion of 1st schedule of intervention 12 subjects again were either found fit to continue intervention or not willing to continue again for 80 days. So, finally 55 subjects completed 2nd schedule of intervention. So dropout rate was found 26.67%. (Figure 2)

Figure 2



These 55 participants were having mean age 38 years with SD 16.19 years with age range 30 years to 59 years. These participants were having mean ROS, 8-OHdG, Cortisol (ng/mL), IL-6(pg/mL),

β – Endorphin (pg/mL), Telomerase (IU/Cell) and Telomere (T/S) level 1215.069 (RLU/min/10^4 Neutrophils), 10268.23 (pg/mL), 118.83 (ng/mL), 2.29 (pg/mL), 3.23 (pg/mL), 0.59 (TU/Cell) and 0.636 (T/S) respectively.

It was observed from this study that there was a significant reduction in oxidative stress markers such as ROS and 8-OHdG, the psychological stress markers as plasma cortisol and IL-6. Although Telomere length did not change up to significant label after interventions but significant increase in telomerase and β – endorphin levels at day 0 vs.10 days; 0 day vs. 90 days and 10 days vs. 90 days was also observed in this study. (Table 1)

Table 1

Comparison of various parameters at Base level and 10^{th} & 90^{th} Day after intervention

S. No.	Variables	Baseline and Post Intervention (Mean ± S.D)			Significance Level with 'P' Value		
		At '0' day (A)	on 10^{th} day (B)	90^{th} day (C)	(A) Vs (B)	(A) Vs (C)	(B)Vs (C)
1	ROS (RLU/Min/10^4 N)	1215.07 ± 0.9	1020.81 ± 0.79	905.81 ± 31.79	0.024*	0.01**	0.047*
2	8-OHdG (pg/mL)	10268.2±3349	9367.6 ± 2709.6	6367.6 ± 578.2	0.159	0.041*	0.047*
3	Cortisol (ng/mL)	118.83 ± 30.58	96.32 ± 36.06	91.32 ± 19.2	0.007*	0.02*	0.425
4	IL-6(pg/mL)	2.29 ± 0.32	1.99 ± 0.11	1.91 ± 0.17	0.036*	0.029*	0.162
5	β – Endorphin (pg/mL)	3.23 ± 0.86	7.08 ± 0.61	11.31± 0.35	0.021*	0.001***	0.01**
6	Telomerase (IU/Cell)	0.59± 0.114	1.98 ± 0.568	32±11.03	0.041*	0.01*	0.001***
7	Telomere (T/S)	0.636±0.079	0.630±0.059	0.619±0.03	0.091	0.057	0.061

4. Discussion

This present study found that these lifestyle interventions (yoga/meditation) may cause a significant decrease in markers of oxidative stress (ROS levels) and this is evident as the oxidative DNA damage marker (8-OHdG) also reduced significantly. Few findings suggest that telomeres are prone to damage by oxidative stress due to the presence of high numbers of guanine residues[15,16,17] and also, it was proposed previously that the oxidative damage to the nitrogen bases have been contribute severely to the process of aging and senescence.[18] Furthermore, the increased ROS levels are responsible for single-strand breaks in nuclear and mitochondrial DNA, either directly or indirectly and the increased ROS level also hinders the repair process of oxidative bases of DNA. In contrast to the genomic DNA, the telomereic DNA was proposed to be deficient in the repair process of single-strand breaks[19] and this may be due to the insufficient level of telomerase in the aging cells. The results of this study also suggest that the benefits through a positive lifestyle intervention can be evident as early as 10 days even the activity of telomerase also elevated significantly.

Broadly, the aging process may be divided into two groups: aging associated with damage accumulation and developmentally programmed aging. However, an emerging hypothesis insists over the free radical theory that is damage accumulation theory of aging.[20] In this study we pointedly

specified in our data that yoga and meditation might reduce the level of oxidative DNA damage accumulation biomarker (8OHdG) up to normal.[21] Also, in accordance with our study which demonstrates that elevated level of cortisol may also play a role in diminishing the activity of telomerase and ultimately reducing the telomere length, emerging trend also insisted that telomeres shorten with exposure to psychosocial stress including early-life stress.[22] Some reports explain that telomere shortening and activity of telomerase might be one of the best biomarker for the overall stress response of an organism to various pathogenic conditions[23] our findings are also in line. Furthermore, shortened telomere length and reduced telomerase activity are correlated with early mortality as well as a host of health risks[24] which might be controlled partially by psychological stress.[4] However, increased telomerase activity reported to promote immune cell longevity as well.[25] Our findings also demonstrate that elevated activity of telomerase might cause the reduced levels of inflammatory marker IL6 post intervention (even in day 10). Keeping these points in view we may specifically demonstrate that yoga and mediation intervention may also act as anti-inflammatory activity. Very consistent studies are required including this one, will definitely help to answer whether the yoga and meditation regulate these biomarkers if so then the inventions will have broad utility in psychiatry and even may act as medicine for psychosocial stressors. Also, there are possibilities that telomere shortening might be slowed down or reversed by yoga and meditation interventions, could provide an opportunity for translating novel preventative and therapeutic approaches.

Oxidative stress and inflammation contributes in causation, and progression of several life style related chronic diseases. Yoga/meditation is one such practice that combines a healthy lifestyle with mental peace,[6] similar to mind–body medicine functioning on the principles of psycho neuroimmunology[14] A modification in lifestyle and relaxation practices including yoga and meditation were shown to improve clinical profile of patients with various pathologies and enhance immunity[15] and this benefit was independent of the type of clinical diagnosis.

Also, the data regarding the efficacy of lifestyle intervention in this study lasting less than 3 months confirm that yoga/meditation can reduce oxidative stress both by reduction of free radical levels and up regulation of total anti oxidant capacity. Keeping this in view and simple-to-follow yoga and meditation based lifestyle intervention can significantly reduce oxidative stress and inflammation, up regulation of telomerase may aid in telomere length maintenance and prevent accelerated telomere shortening and thus aids in maintenance of chromosomal stability and maintain genomic.

CONCLUSIONS

In the recent years, life has become immensely stressful. Adoption of simple healthy habits and lifestyle can profoundly impact health. Our data in this study suggest that yoga/meditation based lifestyle intervention reduced the markers of stress, inflammation and cellular aging. So that it can be concluded that inclusion of yoga/meditation in daily life if very beneficial, so it should be adopted as healthy lifestyle.

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